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Influence of Trace Metals on Some Soil Nitrogen Transformations[1]

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ABSTRACT

An investigation to evaluate the influence of trace metals on N immobilization, N mineralization, and nitrification was conducted. Samples of Yolo silt loam amended with 100 ppm NH_4-N, 1% sewage sludge, and 1% ground alfalfa (*Medicago sativa* L.) were treated with solutions to provide 100, 200, and 400 ppm of Cd(II), Cr(III), Cu(II), Mn(II), Pb(II) and Zn(II), and incubated for 2, 4, 8, and 12-week periods. Metals were determined by extracting with water, KNO_3, DTPA (diethylenetriaminepentaacetic acid), and HNO_3 at the end of each incubation period.

All metals reverted quickly to more insoluble forms. Quantities of metals extractable with water + KNO_3 + DTPA decreased with time, while corresponding increases in HNO_3-soluble forms occurred. Nitrogen immobilization was measured during the 2- to 4-week period, and N mineralization and nitrification during the 4- to 12-week period. At low levels of metal addition Mn and Pb stimulated N immobilization, but at 400 ppm all metals were inhibitory to all N transformations. Among the six metals studied the sequence in order of decreasing inhibition was Cr > Cd > Cu > Zn > Mn > Pb. It was concluded that N transformation rates may be affected by trace metals in soils receiving heavy sludge applications.

Additional Index Words: **trace metal toxicity, N immobilization, N mineralization, nitrification.**

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The application of sewage sludge has implications for microbial activities in soils as well as for plant uptake of potentially toxic metals and their introduction into the food chain. The sensitivity of microorganisms to toxic metal ions may vary (Talburt and Johnson, 1967), and some species are known to develop resistance by genetic or phenotypic changes resulting in exclusion or metabolism of the toxic ion (Ashida, 1965). Metal-tolerant organisms have been isolated from soil where high concentrations occur naturally and from soils that have been polluted by heavy metals (Hartman, 1974). Several soil variables may influence the toxic effects of trace metals, including organic matter content, kind and amount of clay, and soil pH (Bunzl et al., 1976; Gadd and Griffiths, 1978; Sinha et al., 1978). Binding of metals to organic materials appears to be particularly important (Bloomfield et al., 1976).

A few workers have investigated the influence of metals in soils on N transformations, and have reported somewhat mixed results. Wilson (1977) investigated nitrification in soil treated with domestic and industrial sewage sludge and concluded that activity of nitrifiers was inhibited significantly by sludge containing high levels of Cd, Pb, and Zn. Premi and Cornfield (1969) found that ammonification was reduced by 10,000 ppm Cu, but 1,000 ppm Cu or 100 ppm Cr had no effect. Quraishi and Cornfield (1973) reported that addition of up to 10,000 ppm Cu stimulated N-mineralization and nitrification during incubation of a sandy loam treated with 200 ppm N as dried blood. The maximum stimulating effect was at 1,000 ppm Cu. On the other hand, Liang and Tabatabai in studying the influence of 19 trace elements on N mineralization (1977) and on nitrification (1978) added at the level of 5 μmol/g soil found inhibitory effects in every instance.

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Table 1—Some properties of the soil and sludge used in the experiment.

Constituent	Soil	Digested sludge
	ppm	
Cd	0.07	2.90
Cr	2.58	66.4
Cu	7.05	256
Mn	295	239
Pb	2.27	88.1
Zn	6.59	448
Organic N	1,180	16,320
NH_4-N	1.4	291
NO_3-N	7.2	9.8
pH	6.9	

The purpose of the investigation reported here was to evaluate the effects of six different trace metals on the processes of N mineralization, N immobilization, and nitrification in a Yolo soil.

MATERIALS AND METHODS

The soil used was Yolo silt loam (Typic Xerorthent) amended with 1% dry digested sewage sludge and 1% ground alfalfa containing 4.42% total N. The purpose of the alfalfa was to stimulate N transformation processes in the soil. In addition, 100 ppm N as $(NH_4)_2SO_4$ was added at the beginning of the experiment. Some properties of the soil and sludge are given in Table 1. Trace metals added to the amended soil samples were analytical reagent-grade chemicals. In concentrations equivalent to 0, 100, 200, and 400 ppm on the dry-soil basis, Cd(II), Cr(III), Mn(II), and Pb(II) were applied as the chloride, and Cu(II) and Zn(II) as the sulfate. The solutions were added dropwise so as to moisten the whole soil sample to 60% of water-holding capacity. Triplicate 50-g soil samples were incubated for 2-, 4-, 8-, and 12-week periods at 25°C in well-aerated flasks. At the end of each incubation period samples were extracted with 1*N* KCl and the extract analyzed for inorganic N by steam distillation with Devarda's alloy (Bremner and Keeney, 1966). Nitrate was determined colorimetrically by the sulfuric-salicylic acid method of Cataldo et al. (1975). The extracted soil was then analyzed for organic N by Kjeldahl digestion. A separate 10-g subsample was extracted with 25 ml of water for 1 hour, 25 ml of 1*M* KNO_3 for 2 hours, 25 ml of DTPA solution for 2 hours, and 25 ml of 1*M* HNO_3 for 2 hours, in that sequence. The DTPA solution was that described by Lindsay and Norvell.[3] Extracts were separated by centrifugation and analyzed for metals by atomic absorption spectroscopy. The purpose of the sequence of extractants was to estimate water-soluble, exchangeable (KNO_3-extractable), available (DTPA-extractable), and residual (HNO_3-extractable) forms of metals.

RESULTS AND DISCUSSION

Cumulative recovery of metals soluble in the sequence of four extractants was less than complete for all metals (Table 2), ranging from 10–13% for Cr to 40–68% for Zn. While addition of water-soluble metals to soil is not comparable to addition of similar levels of metals in sludge, the proportion of added metals that could be extracted with water after 2 weeks incubation was extremely low. Water-soluble plus exchangeable metals, represented by amounts removed by water followed by KNO_3, were likewise very low except in the case of Mn (Table 3). Moreover, values shown in Table 3 are nearly independent of level of metal added. Quantities of metals soluble in water, KNO_3, and DTPA decreased progressively with time, indicating gradual conversion to less soluble forms. The relationship between the decrease in quantities of metals soluble in H_2O + KNO_3 + DTPA and the increase in quantities soluble in HNO_3, using all levels of addition and all four sampling periods gave a regression $Y = 1.03\ X$, with $R = 0.997$. The reversion of metals to less soluble forms between 2 and 12 weeks was described by relationships of the form $\log Y = A + B \log t$, where Y is concentration of metal extractable with HNO_3 and t is time in weeks (Fig. 1). Lead and cadmium decreased in solubility most rapidly, and Cr and Cu the least. The foregoing data indicate that inhibitory effects of metals on N-transformation processes in this system occur at low levels of metal solubility, in spite of the size of the initial additions of metals.

[3] W. L. Lindsay and W. A. Norvell, 1969. Development of a DTPA micronutrient soil test. Agron Abstracts, Am. Soc. Agron., Madison, Wis. p. 84.

Table 2—Percent recovery of added metals from a Yolo soil after 2 weeks of incubation using a sequence of extractions with H_2O, KNO_3, DTPA, and HNO_3.

Metal added	Cd	Cr	Cu	Pb	Zn	Mn
ppm	%					
100	38	10	44	34	40	59
200	27	13	54	28	68	66
400	22	13	52	33	61	56

Table 3—Percent of total metal extracted sequentially from a Yolo soil with water and KNO_3 after 2 weeks of incubation.

Metal added	Cd	Cr	Cu	Mn	Pb	Zn
ppm	%					
100	0.73	0	0.28	17.4	0.23	0.82
200	1.15	0.56	0.32	18.5	0.32	2.43
400	0.95	0.54	0.35	19.4	0.89	1.75

N Transformations

Values of organic (N_o) and inorganic N (N_i) are presented in Table 4. The period from 2 to 4 weeks was one of net N immobilization, since the level of inorganic N decreased and that of organic N increased. During this period the relationship between changes in the two forms of N was described by the equation $\Delta N_o = 3.15 - 0.888\ \Delta N_i$, with a correlation coefficient of -0.986. From 4 to 12 weeks the quantity of organic N decreased, with a corresponding increase of inorganic N, described

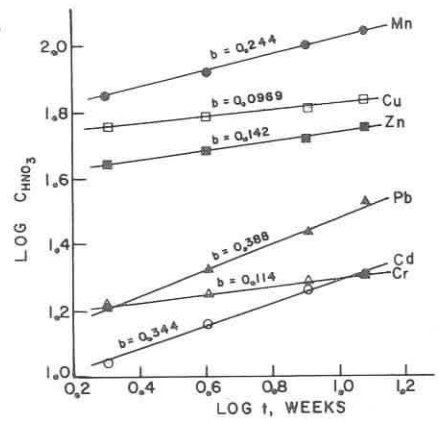

Fig. 1—Relationships between concentrations of HNO_3-soluble metals and time of incubation.

by the relationship $\Delta N_o = 14.2 - 1.32\ \Delta N_i$, with $R = -0.972$. Since both equations indicate a loss of inorganic N from the system, measured values for ΔN_i and ΔNO_3^--N were lower than would otherwise have been found. Nitrate-N concentrations at the 4- and 12-week sampling times are presented in Table 5.

The extent of N loss was estimated from changes in N_t, the sum of $N_o + N_i$. Between 2 and 4 weeks, the period of net immobilization, ΔN_t values were so low as to be disregarded. Between 4 and 12 weeks, the period of net mineralization, ΔN_t values were as reported in Table 6. Nitrogen losses decreased with increasing levels of metal addition. Among individual metals, Cr inhibited N losses the most and Pb the least. Since N losses from the system such as commonly occur in incubation experiments affect estimates of N immobilization and N mineralization based on ΔN_i values, the influence of metals on these processes might be more reliably indicated by ΔN_o values, which, however, can be less precisely determined. As shown in Table 7, the same conclusions can be drawn from both sets of data. For net immobilization (2–4 weeks) each increment of metal produced a significant change in ΔN_o and ΔN_i. Between 4 and 12 weeks 100 ppm of added metal stimulated both N mineralization and NO_3 production and 400 ppm inhibited these processes, while at 200 ppm ΔN_o and ΔNO_3^--N were not significantly different from the control.

Effects of individual metals are shown in Table 8. For both N immobilization and mineralization the sequence of metals is the same in terms of their inhibitory effect, whether one uses the ΔN_o or ΔN_i data. The sequence is Cr > Cd > Cu > Zn > Mn > Pb, although not all means are significantly different.

Table 4—Concentrations of organic N(N_o) and inorganic N(N_i) in a Yolo soil at 2-, 4-, and 12-week sampling dates as affected by metal additions.

Concn. added	Metal added					
	Cd	Cr	Cu	Pb	Zn	Mn
	ppm					
			N_o, 2 weeks			
0	1,280	1,280	1,280	1,280	1,280	1,280
100	1,426	1,451	1,400	1,300	1,376	1,326
200	1,451	1,483	1,436	1,331	1,423	1,360
400	1,470	1,501	1,450	1,363	1,436	1,371
			N_o, 4 weeks			
0	1,361	1,361	1,361	1,361	1,361	1,361
100	1,475	1,496	1,460	1,421	1,541	1,431
200	1,479	1,508	1,481	1,425	1,466	1,421
400	1,496	1,510	1,490	1,414	1,471	1,416
			N_o, 12 weeks			
0	1,241	1,241	1,241	1,241	1,241	1,241
100	1,320	1,366	1,280	1,185	1,251	1,211
200	1,355	1,425	1,351	1,241	1,326	1,271
400	1,421	1,461	1,396	1,286	1,371	1,306
			N_i, 2 weeks			
0	175	175	175	175	175	175
100	91.7	90.5	100	183	122	147
200	67.5	57.5	70.3	153	75.6	105
400	48.6	43.4	50.5	121	63.6	98.2
			N_i, 4 weeks			
0	82.5	82.5	82.5	82.5	82.5	82.5
100	40.5	45.6	38.4	52.7	42.5	36.4
200	34.9	30.4	19.5	45.4	30.5	48.4
400	25.5	29.3	20.4	55.6	28.6	54.3
			N_i, 12 weeks			
0	172	172	172	172	172	172
100	180	163	195	236	211	215
200	148	108	136	185	151	171
400	93.4	80.5	93.7	155	112	141

Standard errors of means	N_o	N_i
Period of incubation, A	2.02	1.23
Concentration of metal added, B	2.33	1.43
Kind of metal, C	2.85	1.75
A × B	4.03	2.47
A × C	4.94	3.02
B × C	5.70	3.49
Replicates treated alike	9.87	6.05

Nitrification

Nitrogen losses from the system are most likely to result from denitrification; thus, the ΔNO_3^--N values of Tables 7 and 8 may not provide an accurate measure of nitrification. Assuming that the losses calculated from ΔN_t values between 4 and 12 weeks can be attributed to denitrification, the values of ΔNO_3^--N can be corrected by addition of the corresponding ΔN_t values. When this was done, the data of Table 9 were obtained. When these are compared with the right-hand columns in

Table 5—Nitrate-N concentrations in a Yolo soil after 4 and 12 weeks incubation as affected by metal additions.

Concn. added	Metal added					
	Cd	Cr	Cu	Pb	Zn	Mn
	ppm					
			4 weeks			
0	79.1	79.1	79.1	79.1	79.1	79.1
100	25.4	27.4	27.7	45.9	31.9	31.0
200	19.0	15.3	13.0	38.3	21.4	39.8
400	10.4	11.3	11.2	43.4	17.2	40.8
			12 weeks			
0	165	165	165	165	165	165
100	112	98.5	141	208	161	185
200	80.8	54.6	89.8	159	107	143
400	38.0	31.1	52.0	123	68.8	107

Standard errors of means	
Time of incubation, A	1.11
Concentration of metal, B	1.58
Kind of metal, C	1.93
A × B	2.23
A × C	2.73
B × C	3.86
Replicates treated alike	5.46

Table 6—Effects of added metals on the loss of N from a Yolo soil between 4 and 12 weeks of incubation as measured by changes in organic plus inorganic N (N_t).

Means of all metals		Means of all concentrations	
Metal added	ΔN_t	Metal	ΔN_t†
ppm			ppm
0	31 c	Cd	11 ab
100	29 bc	Cr	5 a
200	20 ab	Cu	19 abc
400	17 a	Pb	43 d
		Zn	22 bc
		Mn	30 c

† For a given column, means followed by the same letter are not significantly different at the 5% level.

Table 7—Cumulative effects of six metals added to Yolo soil on changes of organic- (N_o), inorganic- (N_i), and NO_3-N.†

Metal added	2-4 weeks		4-12 weeks		
	ΔN_o	ΔN_i	ΔN_o	ΔN_i	ΔNO_3-N
	ppm				
0	81 c	−92.4 d	−120 b	93.7 b	85.6 b
100	76 c	−79.7 c	−187 c	162 d	199 c
200	49 b	−53.4 b	−135 b	199 c	81.2 b
400	34 a	−35.3 a	−93.1 a	81.3 a	47.7 a

† For a given column, means followed by the same letter are not significantly different at the 5% level.

Table 8—Effects of kind of metal on changes of organic-, (N_o), inorganic- (N_i), and NO_3-N; means of all concentrations added.†

Metal added	2-4 weeks		4-12 weeks		
	ΔN_o	ΔN_i	ΔN_o	ΔN_i	ΔNO_3-N
	ppm				
Cd	46 a	−49.6 b	−119 b	103 b	65.4 a
Cr	40 a	−44.7 a	−96 a	84 a	53.9 a
Cu	56 ab	−58.8 c	−131 bc	109 b	79.0 ab
Pb	87 c	−98.9 e	−168 d	128 c	112 c
Zn	58 ab	−63.0 c	−140 c	116 b	88.0 ab
Mn	73 bc	−76.1 d	−150 cd	119 bc	102 bc

† For a given column, means followed by the same letter are not significantly different at the 5% level.

Tables 7 and 8 it will be observed that the inferences to be drawn concerning level of metal addition and nature of metal in their influence on NO_3 production are exactly the same whether corrected or uncorrected data are used. As with the other N transformations, the sequence in terms of inhibitory effects is Cr > Cd > Cu > Zn > Mn > Pb. Appreciable amounts of NH_4-N were found in all metal-treated samples, indicating that nitrification was not retarded by lack of substrate at any time.

No clear relationship was found between toxicity of individual metals to N transformations and solubility in separate extractants. However, a plot of percent inhibition of nitrification against concentrations of metals soluble in H_2O + KNO_3 + DTPA, shown in Fig. 2, gives a series of curves whose slope and position are related to toxicity. Curves for the more toxic elements such as Cr and Cd have very steep slopes and appear at the left of the figure, whereas less toxic metals have less steep slopes and appear to the right. The slopes of all curves decrease with increasing levels of metal addition,

Table 9—Effects of added metals on increase in NO_3 production between 4 and 12 weeks using data corrected for N losses.†

Means for all metals		Means for all levels of addition	
Metal added	ΔNO_3-N	Metal	ΔNO_3-N
ppm			ppm
0	116 b	Cd	81.1 a
100	148 b	Cr	63.3 a
200	101 b	Cu	103 ab
400	64 a	Pb	152 c
		Zn	112 ab
		Mn	133 bc

† For a given column, means followed by the same letter are not significantly different at the 5% level.

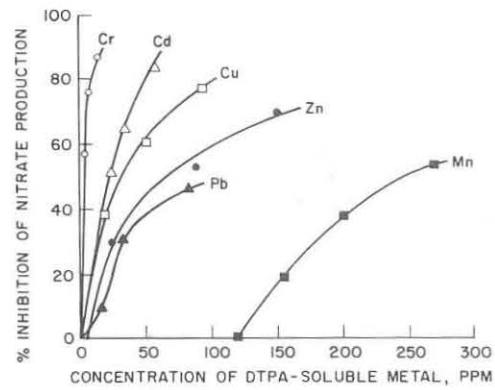

Fig. 2—Relationships between DTPA-soluble metals and percent inhibition of nitrification in Yolo silt loam.

indicating that the first increments of metal are more toxic to the nitrifying bacteria than are subsequent additions.

Although these findings obtained with a single soil do not provide a basis for generalization, the fact that the same toxicity sequence was observed for N immobilization, mineralization, NO_3 production, and N loss suggests that the results may be more widely applicable.

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